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Title: **NOVEL COMPOUNDS**

Reference: F2210-1SE

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NOVEL COMPOUNDS

The present invention provides novel hydroxypyrrolidine compounds, their use as medicaments, compositions containing them and processes for their preparation.

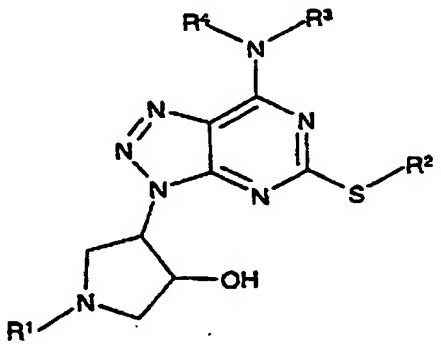
Platelet adhesion and aggregation are initiating events in arterial thrombosis. Although the process of platelet adhesion to the sub-endothelial surface may have an important role to play in the repair of damaged vessel walls, the platelet aggregation that this initiates can precipitate acute thrombotic occlusion of vital vascular beds, leading to events with high morbidity such as myocardial infarction and unstable angina. The success of interventions used to prevent or alleviate these conditions, such as thrombolysis and platelet-mediated occlusion or re-occlusion also compromises angioplasty.

A number of converging pathways lead to platelet aggregation. Whatever the initial stimulus, the final common event is a cross-linking of platelets by binding of fibrinogen to a membrane-binding site, glycoprotein IIb/IIIa (GPIIb/IIIa). The high anti-platelet efficacy of antibodies or antagonists for GPIIb/IIIa is explained by their interference with this final common event. However, this efficacy may also explain the bleeding problems that have been observed with this class of agent. Thrombin can produce platelet aggregation largely independently of other pathways but substantial quantities of thrombin are unlikely to be present without prior activation of platelets by other mechanisms. Thrombin inhibitors such as hirudin are highly effective anti-thrombotic agents, but again may produce excessive bleeding because they function as both anti-platelet and anti-coagulant agents (The TIMI 9a Investigators (1994), *Circulation* **90**, pp. 1624-1630; The Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) IIa Investigators (1994) *Circulation* **90**, pp. 1631-1637; Neuhaus K. L. et. al. (1994) *Circulation* **90**, pp. 1638-1642).

It has been found that ADP acts as a key mediator of thrombosis. A pivotal role for ADP is supported by the fact that other agents, such as adrenaline and 5-hydroxytryptamine (5HT, serotonin) will only produce aggregation in the presence of ADP. The limited anti-thrombotic efficacy of aspirin may reflect the fact that it blocks only one source of ADP which is that released in a thromboxane-dependent manner following platelet adhesion (see e.g. Antiplatelet Trialists' Collaboration (1994), *Br. Med. J.* **308**, pp. 81-106; Antiplatelet Trialists' Collaboration (1994), *Br. Med. J.* **308**, pp. 159-168). Aspirin has no effect on aggregation produced by other sources of ADP, such as damaged cells or ADP released under conditions of turbulent blood flow. ADP-induced platelet aggregation is mediated by the P_{2T} ($P2Y_{ADP}$ or $P2T_{AC}$) -receptor subtype uniquely located on the platelet membrane. Recently it has been shown that antagonists at this

receptor offer significant improvements over other anti-thrombotic agents. Accordingly there is a need to find P_{2T} ($P2Y_{ADP}$ or $P2T_{AC}$) antagonists as anti-thrombotic agents.

In a first aspect the invention therefore provides a compound of formula (I):



(I)

Wherein:

R^1 is H, CH_2R^5 or COR^6;

R^2 is alkyl or alkenyl C_{1-6}, optionally substituted by one or more groups selected from alkyl C_{1-5}, halogen;

R^3 is cycloalkyl C_{3-8}, optionally substituted by R^7;

R^4 is H or alkyl C_{1-6}, optionally substituted by one or more halogens;

R^5 is H, phenyl or alkyl C_{1-6}, optionally substituted by halogen, OR^8, phenyl;

R^6 is OR^8 or alkyl C_{1-6}, optionally substituted by one or more groups selected from halogen, OR^8, phenyl;

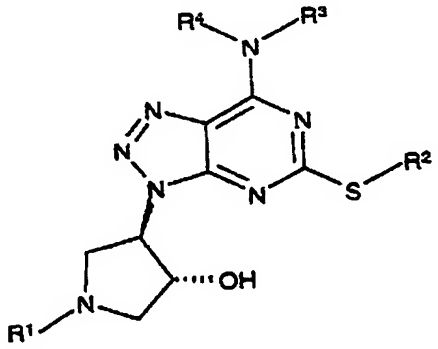
R^7 is phenyl, optionally substituted by one or more groups selected from alkyl C_{1-6}, halogen, OR^8;

R^8 is H or alkyl C_{1-6}, optionally substituted by one or more groups selected from halogen or alkyl C_{1-6};

or a pharmaceutically acceptable salt or solvate thereof.

Compounds of the invention can form pharmaceutically acceptable salts. The compounds of the formula (I) can form acid addition salts with acids, such as conventional pharmaceutically acceptable acids, for example maleic, hydrochloric, hydrobromic, phosphoric, acetic, fumaric, salicylic, citric, lactic, mandelic, tartaric, trifluoroacetic and methanesulphonic acids.

Preferably the compound of formula (I) has the following stereochemistry:



(Ia)

Where R^3 =  the stereochemistry is preferably 

Particularly preferred compounds of the invention include:

[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*S*-[3α,4β(1*S**,2*R**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester;

[3*S*-[3α,4β(1*R**, 2*S**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester;

[3*S*-[3α,4β(1*S**,2*R**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[*N*-Methyl-*N*-(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*R*-[3α,4β(1*R**,2*S**)]]-1-Hydroxyethyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

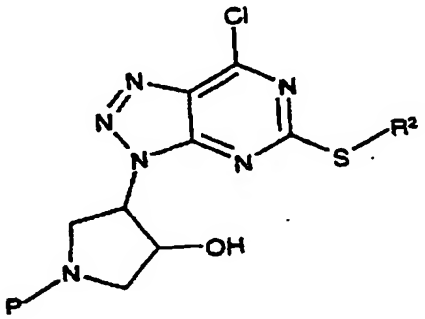
[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-(phenylmethyl)-3-pyrrolidinol;

[3*R*-[3α, 4β(1*R**,2*S**)]]-1-Acetyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol.

or pharmaceutically acceptable salts or solvates thereof.

According to the invention there is further provided a process for the preparation of a compound of formula (I) which comprises:

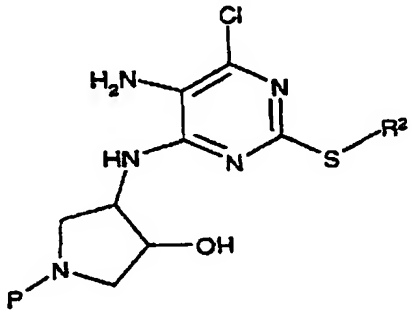
a. For compounds of formula (I) where R^1 is H, reacting a compound of formula (II)



wherein R^2 is as defined above and P is a protecting group, preferably t-BuOCO, with R^3R^4NH, wherein R^3 and R^4 are as defined in (I), and a base, preferably triethylamine or *N,N*-diisopropylethylamine, in the presence of an inert solvent preferably acetonitrile, preferably at a temperature between 20 °C and 100 °C and optionally thereafter removing any protecting groups.

Protecting groups can be added and removed using known reaction conditions. The use of protecting groups is fully described in 'Protective Groups in Organic Chemistry', edited by J W F McOmie, Plenum Press (1973), and 'Protective Groups in Organic Synthesis', 2nd edition, T W Greene & P G M Wutz, Wiley-Interscience (1991).

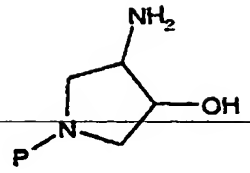
A compound of formula (II) can be prepared by diazotizing a compound of formula (III)



(III)

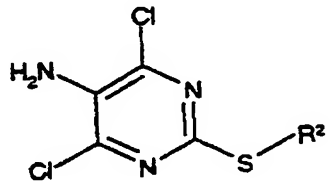
where R^2 and P are defined above, and where necessary other reactive groups might also be protected, with a C_{1-6} alkyl nitrite, preferably iso-amylnitrite in the presence of an inert solvent prefcrably acetonitrile at 20-80°C, or with an alkali metal nitrite, preferably sodium nitrite, under aqueous acidic conditions, preferably aqueous hydrochloric or acetic acid and preferably at a temperature between 0°C and 20°C.

A compound of formula (III) can be prepared by reacting a compound of formula (IV).



(IV)

wherein P is a protecting group, with a compound of formula V

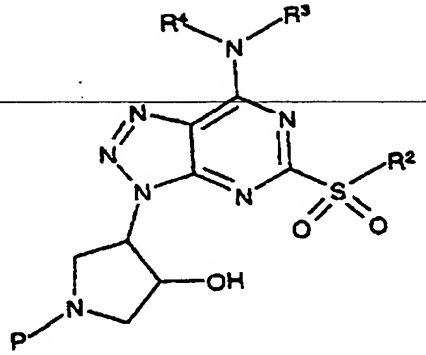


(V)

wherein R^2 is as defined in formula (I) and is preferably n-propyl. The reaction is carried out in the presence of a base, preferably triethylamine or *N,N*-disopropylethylamine, in an inert solvent preferably *N,N*-dimethylformamide or n-butanol, at a temperature between 100°C and 150°C.

The preparation of the formula (IV) racemate is described in Okada et al., Chem. Pharm. Bull. (1993), 41, 132-8; the preparation of formula (IV) enantiomers is described in Schaus, et al., J. Org. Chem. (1997), 62, 4197-9; the preparation of a compound of formula V (R^2 is n-propyl) is described in EP 508687.

Compounds of formula (I) where R^2 is other than n-propyl are prepared by displacement of the sulphone group from a compound of formula (VI)



(VI)

where R^2 is n-propyl, P, R^3 and R^4 are defined above, using either a sodium alkylthiolate (R^2SNa) in the presence of an inert solvent, preferably *N,N*-dimethylformamide, preferably at a temperature between 0 °C and 50 °C or sodium hydrosulphide (NaSH), in the presence of an inert solvent preferably *N,N*-dimethylformamide. This reaction is followed by alkylation with an alkyl halide (R^2X, where X is a leaving group prefcrably bromide or iodide), preferably at a temperature between 0 °C and 50 °C and optionally thereafter removing any protecting groups.

The preparation of the compound of formula (VI), where R^2 is n-propyl, is preferably carried out by reacting a compound of formula (I), where R^1 has been protected as described above, with a peracid, preferably *m*-chloroperbenzoic acid, in the presence of an inert chlorocarbon solvent such as dichloromethane or a mixture of dichloromethane and methanol, at a temperature between 0 °C and 50 °C.

b. For compounds of formula (I) where R^1 is CH_2R^5, where R^5 is defined in formula (I), the reaction scheme outlined in a. above is followed by reductive amination using an aldehyde (R^5CHO) and a reducing agent, preferably sodium triacetoxyborohydride, and optionally thereafter removing any protecting groups. The reductive amination reaction is preferably carried out in the presence of an inert solvent preferably *N,N*-dimethylformamide, tetrahydrofuran or a mixture of acetonitrile and *N*-methylpyrrolidone and preferably at a temperature between 0 °C and 50 °C.

c. For compounds of formula (I) where R^1 is COR^6, where R^6 is defined in formula (I), the reaction scheme outlined in a. above is followed by acylation using an acid halide (R^6COX) or anhydride ($(R^6CO)_2O$) or an acid (R^6CO_2H) in the presence of a suitable activating agent preferably *N,N'*-carbonyldiimidazole or *N,N'*-dicyclohexylcarbodiimide, and a base preferably triethylamine or *N,N*-disopropylethylamine, and optionally thereafter removing any protecting groups. The acylation is preferably carried out in the presence of an inert solvent preferably dichloromethane, chloroform or tetrahydrofuran and preferably at a temperature between 0 °C and 50 °C.

All novel intermediates form a further aspect of the invention.

Salts of the compounds of formula (I) may be formed by reacting the free base, or a salt or a derivative thereof, with one or more equivalents of the appropriate acid (for example a hydrohalic (especially HCl), sulphuric, oxalic or phosphoric acid). The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, e.g. water, ethanol, THF or diethyl ether, which may be removed *in vacuo*, or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange resin. The non-toxic physiologically acceptable salts are preferred, although other salts may be useful, e.g. in isolating or purifying the product.

The compounds of the invention act as P_{2T} ($P2Y_{ADP}$ or $P2T_{AC}$) receptor antagonists. Accordingly, the compounds are useful in therapy, including combination therapy, particularly they are indicated for use as: inhibitors of platelet activation, aggregation and degranulation, promoters of platelet disaggregation, anti-thrombotic agents or in the treatment or prophylaxis of unstable angina, coronary revascularisation procedures including angioplasty (PTCA), myocardial infarction, perithrombolysis, primary arterial thrombotic complications of atherosclerosis such as thrombotic or embolic stroke, transient ischaemic attacks, peripheral vascular disease, myocardial infarction with or without thrombolysis, arterial complications due to interventions in atherosclerotic disease such as angioplasty, endarterectomy, stent placement, coronary and other vascular graft surgery, thrombotic complications of surgical or mechanical damage such as tissue salvage following accidental or surgical trauma, reconstructive surgery including skin and muscle flaps, conditions with a diffuse thrombotic/platelet consumption component such as disseminated intravascular coagulation, thrombotic thrombocytopaenic purpura, haemolytic uraemic syndrome, thrombotic complications of septicaemia, adult respiratory distress syndrome, anti-phospholipid syndrome, heparin-induced thrombocytopaenia and pre-eclampsia/eclampsia, or venous thrombosis such as deep vein thrombosis, venoocclusive disease, haematological conditions such

as myeloproliferative disease, including thrombocythaemia, sickle cell disease; or in the prevention of mechanically-induced platelet activation *in vivo*, such as cardio-pulmonary bypass and extracorporeal membrane oxygenation (prevention of microthromboembolism), mechanically-induced platelet activation *in vitro*, such as use in the preservation of blood products, e.g. platelet concentrates, or shunt occlusion such as in renal dialysis and plasmapheresis, thrombosis secondary to vascular damage/inflammation such as vasculitis, arteritis, glomerulonephritis, inflammatory bowel disease and organ graft rejection, conditions such as migraine, Raynaud's phenomenon, conditions in which platelets can contribute to the underlying inflammatory disease process in the vascular wall such as atheromatous plaque formation/progression, stenosis/restenosis and in other inflammatory conditions such as asthma, in which platelets and platelet-derived factors are implicated in the immunological disease process. Further indications include treatment of CNS disorders and prevention of the growth and spread of tumours.

According to the invention there is further provided the use of a compound according to the invention in the manufacture of a medicament for the treatment of the above disorders. In particular the compounds of the invention are useful for treating myocardial infarction, thrombotic stroke, transient ischaemic attacks, peripheral vascular disease and angina, especially unstable angina. The invention also provides a method of treatment of the above disorders which comprises administering to a patient suffering from such a disorder a therapeutically effective amount of a compound according to the invention.

The compounds may be administered topically, e.g. to the lung and/or the airways, in the form of solutions, suspensions, HFA aerosols and dry powder formulations; or systemically, e.g. by oral administration in the form of tablets, pills, capsules, syrups, powders or granules, or by parenteral administration in the form of sterile parenteral solutions or suspensions, by subcutaneous administration, or by rectal administration in the form of suppositories or transdermally.

The compounds of the invention may be administered on their own or as a pharmaceutical composition comprising the compound of the invention in combination with a pharmaceutically acceptable diluent, adjuvant or carrier. Particularly preferred are compositions not containing material capable of causing an adverse, e.g. an allergic, reaction.

Dry powder formulations and pressurised HFA aerosols of the compounds of the invention may be administered by oral or nasal inhalation. For inhalation the compound is desirably finely divided. The compounds of the invention may also be administered by means of a dry powder inhaler. The inhaler may be a single or a multi dose inhaler, and may be a breath actuated dry powder inhaler.

One possibility is to mix the finely divided compound with a carrier substance, .g. a mono-, di- or polysaccharide, a sugar alcohol or another polyol. Suitable carriers include sugars and starch. Alternatively the finely divided compound may be coated by another substance. The powder mixture may also be dispensed into hard gelatine capsules, each containing the desired dose of the active compound.

Another possibility is to process the finely divided powder into spheres which break up during the inhalation procedure. This spheronized powder may be filled into the drug reservoir of a multidose inhaler, e.g. that known as the Turbuhaler® in which a dosing unit meters the desired dose which is then inhaled by the patient. With this system the active compound with or without a carrier substance is delivered to the patient.

The pharmaceutical composition comprising the compound of the invention may conveniently be tablets, pills, capsules, syrups, powders or granules for oral administration; sterile parenteral or subcutaneous solutions, suspensions for parenteral administration or suppositories for rectal administration.

For oral administration the active compound may be admixed with an adjuvant or a carrier, e.g. lactose, saccharose, sorbitol, mannitol, starches such as potato starch, corn starch or amylopectin, cellulose derivatives, a binder such as gelatine or polyvinylpyrrolidone, and a lubricant such as magnesium stearate, calcium stearate, polyethylene glycol, waxes, paraffin, and the like, and then compressed into tablets. If coated tablets are required, the cores, prepared as described above, may be coated with a concentrated sugar solution, which may contain e.g. gum arabic, gelatine, talcum, titanium dioxide, and the like. Alternatively, the tablet may be coated with a suitable polymer dissolved either in a readily volatile organic solvent or an aqueous solvent.

For the preparation of soft gelatine capsules, the compound may be admixed with e.g. a vegetable oil or polyethylene glycol. Hard gelatine capsules may contain granules of the compound using either the above mentioned excipients for tablets, e.g. lactose, saccharose, sorbitol , mannitol, starches, cellulose derivatives or gelatine. Also liquid or semisolid formulations of the drug may be filled into hard gelatine capsules.

Liquid preparations for oral application may be in the form of syrups or suspensions, for example solutions containing the compound, the balance being sugar and a mixture of ethanol, water, glycerol and propylene glycol. Optionally such liquid preparations may contain colouring agents,

flavouring agents, saccharine and carboxymetbylcellulose as a thickening agent or other excipients known to those skilled in art.

The invention is illustrated by the following examples. In the examples the NMR spectra were measured on a Varian Unity Inova 300 or 400 spectrometer and the MS spectra were measured as follows: EI spectra were obtained on a VG 70-250S or Finnigan Mat Incos-XL spectrometer, FAB spectra were obtained on a VG70-250SEQ spectrometer, ESI and APCI spectra were obtained on Finnigan Mat SSQ7000 or a Micromass Platform spectrometer. Preparative HPLC separations were generally performed using a Novapak®, Bondapak® or Hypersil® column packed with BDSC-18 reverse phase silica. Flash chromatography (indicated in the Examples as (SiO_2)) was carried out using Fisher Matrix silica, 35-70 μm. For examples which show the presence of rotamers in the proton NMR spectra only the chemical shifts of the major rotamer are quoted.

Example 1

[3*R*-[3α,4β(1*R,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

a) (3*R*,4*R*)-3-[[5-Amino-6-chloro-2-(propylthio)pyrimidin-4-yl]amino]-4-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

Triethylamine (18.8ml) was added to a solution of (3*R*,4*R*)-4-amino-3-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester (prepared as described in J. Org. Chem., **1997**, *62*, 4197 using the (*S*,*S*)(salen)Cr(III)complex) (3.63g) and 4,6-dichloro-2-propylthiopyrimidine-5-amine (prepared as described in *EP508687*) (3.56g) and the resulting mixture was heated at 100°C for 24 hours. The excess triethylamine was removed *in vacuo* and the residue was diluted with water and extracted with ethyl acetate. The organic phase was washed with brine, dried and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, dichloromethane:methanol, 97:3 as eluant) followed by trituration with diethylether/isohexane to give the subtitle compound (4.16g).

MS (APCI) 404 ($M+H^+$, 100%).

b) (3*R*,4*R*)-4-[7-Chloro-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

The product from step a) (4.1g) and iso-amylnitrite (2.74ml) were heated under reflux in acetonitrile (20ml) for 1 hour. The reaction mixture was concentrated *in vacuo* and the residue purified by chromatography (SiO_2, ethyl acetate:isohexane, 1:4 as eluant) to afford the sub-title compound (3.32g).

MS (APCI) 415 ($M+H^+$, 100%).

c) [3*R*-[3α,4β(1*R,2*S**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester**

N,N-Diisopropylethylamine (3ml) was added to a solution of the product from step b) (1.2g) and (1*R*-*trans*)-2-phenylcyclopropanamine, [*R*-(*R**, *R**)]-2,3-dihydroxybutanedioate (1:1) (prepared as described by L. A. Mitscher *et al.*, J. Med. Chem., 1986, 29, 2044) (1.23g) in dichloromethane (40ml). The reaction mixture was stirred at room temperature for 16 hours then washed with water. The organic phase was washed with dilute hydrochloric acid and brine, dried and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, dichloromethane:methanol, 99:1 as eluant) to afford the sub-title compound (1.12g).

MS (APCI) 512 ($M+H^+$, 100%).

d) [3*R*-[3α,4β(1*R,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

The product from step c) (0.54g) was dissolved in trifluoroacetic acid (22.5ml) and water (2.5ml) and the solution stirred at room temperature for 4h. The solvents were evaporated and

the residue dried by azeotropic distillation with toluene (4x50ml) followed by methanol (50ml) to give a yellow foam. The crude product was triturated with ether (50ml) to afford a white powder that was recrystallised (ethyl acetate) to afford the title compound (0.37g) as a white solid.

MS (APCI) 412 ($M+H^+$, 100%)

NMR δH (d_6-DMSO) 9.5 (2H, br s), 9.47 (1H, d), 7.10-7.35 (5H, m), 6.28 (1H, d), 5.26 (1H, br m), 4.65 (1H, br s), 3.90 (2H, m), 3.52 (1H, d,AB), 3.3 (1H, m), 3.24. (1H, m), 2.8-3.0 (2H, t,AB), 2.13 (1H, m), 1.54 (1H, d,t), 1.47 (2H, sext.), 1.34 (1H, br q), 0.79 (3H, t).

Example 2

[3*S*-[3α,4β(1*S,2*R**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester**

a) (3*S*,4*S*)-3-[[5-Amino-6-chloro-2-(propylthio)pyrimidin-4-yl]amino]-4-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

Prepared according to the method of Example 1, step a) using (3*S*,4*S*)-4-amino-3-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester (prepared as described in J. Org. Chem., 1997, 62, 4197 using the (*R*,*R*)(salen)Cr(III)complex).

MS (APCI) 404/406 ($M+H^+$), 404 (100%).

b) (3*S*,4*S*)-4-[7-Chloro-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-hydroxy-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

Prepared according to the method of Example 1, step b).

MS (APCI) 315 ($M+H-BOC^+$, 100%).

c) [3S-[3α,4β(1S*,2R*)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

Prepared according to the method of Example 1, step c).

MS (APCI) 512 ($M+H^+$, 100%).

NMR δH (d_6-DMSO) 9.40 (1H, d), 7.31-7.27 (2H, m), 7.20-7.15 (3H, m), 5.78-5.76 (1H, m), 5.11-5.06 (1H, m), 4.61-4.56 (1H, m), 3.94-3.81 (2H, m), 3.69-3.62 (1H, m), 3.30-3.18 (2H, m), 3.11-2.80 (2H, m), 2.15-2.10 (1H, m), 1.73-1.23 (13H, m), 0.80 (3H, t).

Example 3

[3S-[3α,4β(1R*, 2S*)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

a) [3S-[3α,4β(1R*, 2S*)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester

Prepared according to the method of Example 2, step c) using (1*S-trans*)-2-phenyl-cyclopropanamine, [*S*-(*R**, *R**)]-2,3-dihydroxybutanedioate (1:1) (prepared as described by L. A. Mitscher *et al.*, J. Med. Chem., **1986**, *29*, 2044).

MS (APCI) 512 ($M+H^+$, 100%).

NMR δH (d_6-DMSO) 9.40 (1H, d), 7.31-7.27 (2H, m), 7.20-7.15 (3H, m), 5.78-5.76 (1H, m), 5.11-5.06 (1H, m), 4.62-4.58 (1H, m), 3.94-3.81 (2H, m), 3.69-3.63 (1H, m), 3.30-3.18 (2H, m), 3.11-2.80 (2H, m), 2.15-2.11 (1H, m), 1.72-1.23 (13H, m), 0.80 (3H, t).

Example 4

[3S-[3α,4β(1S*,2R*)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthi)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt

a) **[3S-[3α,4β(1S*,2R*)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

Prepared according to the method of Example 1, step d) using the compound of Example 2, step c)

MS (APCI) 412 ($M+H^+$, 100%)

NMR δH (d_6-DMSO) 9.5 (2H, br s), 9.48 (1H, d), 7.10-7.35 (5H, m), 6.30 (1H, d), 5.26 (1H, br m), 4.64 (1H, br s), 3.9 (2H, m), 3.5 (1H, d,AB), 3.26 (1H, m), 3.24. (1H, m), 2.7-3.0 (2H, t,AB), 2.11 (1H, m), 1.55 (1H, d,t), 1.46 (2H, sext.), 1.34 (1H, br q), 0.78 (3H, t).

Example 5

[3R-[3α,4β(1R*,2S*)]]-4-[7-[*N*-Methyl-*N*-(2-phenylcyclopropyl)amino]-5-(propylthio)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt

a) **[3R-[3α,4β(1R*,2S*)]]-3-Hydroxy-4-[7-[N-methyl-*N*-(2-phenylcyclopropyl)amino]-5-(propylthio)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester.**

N,N-Diisopropylethylamine (0.5ml) was added to a solution of the product from Example 1 step b) (0.3g) and (1*R-trans*)-*N*-methyl-2-phenylcyclopropylamine hydrochloride (prepared as described by C. Kaiser *et al*, J. Org. Chem., **1962**, 27, 768-773, using (1*R-trans*)-2-phenylcyclopropanamine, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (prepared as described by L.A. Mitscher *et al*, J. Med. Chem., **1986**, 29, 2044) (0.2g) in dichloromethane (20ml). The

reaction mixture was stirred at room temperature for 48 hours then washed with water. The organic phase was washed with dilute hydrochloric acid and brine, dried and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, dichloromethane:methanol, 99:1 as eluant) to afford the sub-title compound (0.36g).

MS (APCI) 470 ($M+H^+$, 100%).

b) [3*R*-[3α,4β(1*R,2*S**)]]-4-[7-[*N*-Methyl-*N*-(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

A solution of the product from step a) (0.36g) in 9:1 trifluoroacetic acid:water (10ml) was stirred at room temperature for 2 hours. The solvent was removed and co-evaporated with toluene (3x). The residue was dissolved in water (20ml) and ethanol (1ml) and freeze-dried for 16 hours to give the title compound (0.33g).

MS (APCI) 426 ($M+H^+$, 100%).

NMR δH (d_6-DMSO) 9.33 (2H, br s), 7.29 (2H, m), 7.20 (3H, m), 6.04 (1H, br s), 5.27 (1H, m), 4.72 (1H, d), 3.84-3.97 (2H, m), 3.56 (4H, m), 3.31 (1H, d), 3.06 (3H, under DMSO), 2.43 (1H, under H_2O), 1.54-1.66 (3H, m), 1.45 (1H, m), 0.94 (3H, t).

Example 6

[3*R*-[3α,4β(1*R,2*S**)]]-1-Hydroxyethyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

a) [3*R*-[3α,4β(1*R,2*S**)]]-1-[2-[(1,1-Dimethylethyl)(dimethyl)silyl]oxy]ethyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol.**

[[(1,1-Dimethylethyl)dimethylsilyl]oxy]acetaldehyde (*Tet. Lett.*, 1995, *36*, 6033) (0.27g) was added to a solution of the product from Example1 step d) (0.4g) and sodium triacetoxyborohydride (0.48g) in dry tetrahydrofuran (10ml) and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with water and extracted with ethyl acetate (thrice). The combined organic phase was washed with brine, dried and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, dichloromethane:methanol, 99:1 as eluant) to give the sub-title compound (0.2g).

MS (APCI) 570 ($M+H^+$, 100%).

b) [3*R*-[3α,4β(1*R,2*S**)]]-1-Hydroxyethyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol, trifluoroacetate salt**

Tetrabutylammonium fluoride hydrate (0.2g) was added to a solution of the product from step a) (0.2g) in dry tetrahydrofuran (10ml) and the mixture was stirred at room temperature for 16hours. The solvent was removed *in vacuo* and the residue was purified by chromatography (SiO_2, dichloromethane:methanol, 95:5 as eluant). Trifluoroacetic acid (22µl) was added to a solution of the resulting oil in diethylether (5ml) and the solid formed was collected by filtration to give the title compound (0.12g).

MS (APCI) 456 ($M+H^+$, 100%).

NMR δH (d_6-DMSO+D_2O) 7.31 (2H, m), 7.21 (3H, m), 5.36 (1H, br s), 4.87 (1H, br s), 4.18 (1H, m), 4.04 (1H, m), 3.82 (3H, m), 3.55 (1H, under H_2O), 3.45 (2H, m), 3.29(1H, br s), 3.02 (2H, br s), 2.22 (1H, br s), 1.58 (2H, br s), 1.50 (1H, m), 1.36 (1H, m), 0.88 (3H, br s).

Example 7

[3*R*-[3α,4β(1*R,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthi)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-(phenylmethyl)-3-pyrrolidinol, trifluoroacetate salt**

Benzaldehyde (0.1ml) was added to a solution the product from Example 1 step d) (0.26g) and sodium triacetoxyborohydride (0.32g) in dry tetrahydrofuran (10ml) and the mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with water and extracted with ethyl acetate (thrice). The combined organic phase was washed with brine, dried and concentrated. Trifluoroacetic acid (20µl) was added to a solution of the resulting oil in diethylether (5ml) and the solvent was removed *in vacuo*. The residue was dissolved in water (20ml) and ethanol (5ml) and freeze-dried for 16 hours. Purification by chromatography (HPLC, Novapak® C18 column, 0.1% aqueous trifluoroacetic acid:acetonitrile, gradient elution 75:25 to 0:100 over 15 minutes), followed by freeze drying gave the title compound (0.094g).

MS (APCI) 502 ($M+H^+$, 100%).

NMR δH (d_6-DMSO+D_2O) 7.53 (2H, d), 7.48 (3H, m), 7.31 (2H, m), 7.20 (3H, m), 5.34 (1H, m), 4.88 (1H, m), 4.48 (2H, q), 4.05 (1H, m), 3.90 (1H, m), 3.72 (1H, m), 3.41 (1H, m), 3.30(1H, br m), 3.01 (2H, br m), 2.21 (1H, br s), 1.50-1.56 (3H, m), 1.36 (1H, m), 0.87 (3H, br s).

Example 8

[3*R*-[3α, 4β(1*R,2*S**)]]-1-Acetyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol.**

A mixture the product from Example 1 step d) (0.17g), acetic anhydride (0.046ml) and pyridine (0.078ml) in dichloromethane (3ml) was stirred at room temperature under a nitrogen atmosphere for 16 hours. The reaction mixture was diluted with water and extracted with

dichloromethane (twice). The combined organic phase was washed with dilute hydrochloric acid and brine, dried and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, dichloromethane:methanol, 98:2 as eluant) followed by trituration with acetonitrile to give the title compound (0.06g).

MS (APCI) 454 ($M+H^+$, 100%).

NMR δH (d_6-DMSO) 9.39 (1H, m), 7.30 (2H, m), 7.19 (3H, m), 5.77-5.86 (1H, m), 5.09-5.16 (1H, m), 4.60-4.69 (1H, m), 4.00-4.13 (1H, m), 3.91 (2H, m), 3.46, 3.68 (1H, m), 3.21 (1H, br m), 2.82-2.91 (2H, m), 2.13 (1H, m), 1.98 (3H, d), 1.34-1.54 (4H, m), 0.79 (3H, t).

Pharmacological data

The preparation for the assay of the P_{2T} ($P2Y_{ADP}$ or $P2T_{AC}$)-receptor agonist/antagonist activity in washed human platelets for the compounds of the invention was carried out as follows.

Human venous blood (100 ml) was divided equally between 3 tubes, each containing 3.2% trisodium citrate (4 ml) as anti-coagulant. The tubes were centrifuged for 15 minutes at 240G to obtain a platelet-rich plasma (PRP) to which 300 ng/ml prostacyclin was added to stabilize the platelets during the washing procedure. Red cell free PRP was obtained by centrifugation for 10 minutes at 125G followed by further centrifugation for 15 minutes at 640G. The supernatant was discarded and the platelet pellet resuspended in modified, Calcium Free Tyrode solution (10 ml) (CFT), composition: NaCl 137mM, $NaHCO_3$ 11.9mM, NaH_2PO_4 0.4mM, KCl 2.7 mM, $MgCl_2$ 1.1 mM, dextrose 5.6 mM, gassed with 95% O_2/5% CO_2 and maintained at 37°C. Following addition of a further 300 ng/ml PGI_2, the pooled suspension was centrifuged once more for 15 minutes at 640G. The supernatant was discarded and the platelets resuspended initially in 10 ml CFT with further CFT added to adjust the final platelet count to $2x10^5$/ml. This final suspension was stored in a 60 ml syringe at 3°C with air excluded. To allow recovery from PGI_2-inhibition of normal function, platelets were used in aggregation studies no sooner than 2 hours after final resuspension.

In all studies, 3 ml aliquots of platelet suspension were added to tubes containing $CaCl_2$ solution (60 µl of 50 mM solution with a final concentration of 1mM). Human fibrinogen (Sigma, F 4883) and 8-sulphophenyltheophylline (8-SPT which was used to block any P_1-agonist activity of compounds) were added to give final concentrations of 0.2 mg/ml (60 µl of 10 mg/ml solution of clottable protein in saline) and 300 nM (10 µl of 15 mM solution in 6% glucose), respectively. Platelets or buffer as appropriate were added in a volume of 150 µl to the individual wells of a 96 well plate. All measurements were made in triplicate in platelets from each donor.

The agonist/antagonist potency was assessed as follows.

Aggregation responses in 96 well plates were measured using the change in absorbance given by the plate reader at 660 nm. Either a Bio-Tec Ceres 900C or a Dynatech MRX were used as the plate reader.

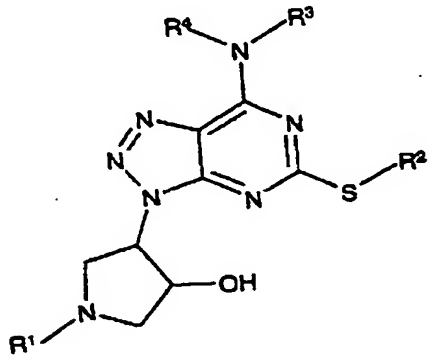
The absorbance of each well in the plate was read at 660 nm to establish a baseline figure. Saline or the appropriate solution of test compound was added to each well in a volume of 10 µl to give a final concentration of 0, 0.01, 0.1, 1, 10 or 100 mM. The plate was then shaken for 5 min on an

orbital shaker on setting 10 and the absorbance read at 660 nm. Aggregation at this point was indicative of agonist activity of the test compound. Saline or ADP (30 mM; 10 µl of 450 mM) was then added to each well and the plate shaken for a further 5 min before reading the absorbance again at 660 nm.

Antagonist potency was estimated as a % inhibition of the control ADP response to obtain an IC_{50}. Compounds exemplified have pIC_{50} values of more than 5.0.

Claims

1. A compound of formula (I):



(I)

Wherein:

R^1 is H, CH_2R^5 or COR^6;

R^2 is alkyl or alkenyl C_{1-6}, optionally substituted by one or more groups selected from alkyl C_{1-6}, halogen;

R^3 is cycloalkyl C_{3-8}, optionally substituted by R^7;

R^4 is H or alkyl C_{1-6}, optionally substituted by one or more halogens;

R^5 is H, phenyl or alkyl C_{1-6}, optionally substituted by halogen, OR^8, phenyl ;

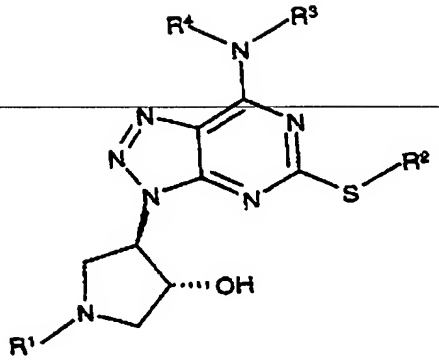
R^6 is OR^8 or alkyl C_{1-6}, optionally substituted by one or more groups selected from halogen, OR^8, phenyl;

R^7 is phenyl, optionally substituted by one or more groups selected from alkyl C_{1-6}, halogen, OR^8;

R^8 is H or alkyl C_{1-6}, optionally substituted by one or more groups selected from halogen or alkyl C_{1-6};

or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to claim 1 having the following stereochemistry:



(Ia)

Where R^3 =  the stereochemistry is preferably 

or a pharmaceutically acceptable salt or solvate thereof.

3. A compound according to claim 1 or 2 in which R^1 is H, CH_2Ph, CH_2CH_2OH, or CO_2tBu.

4. A compound according to claim 1 or 2 in which R^2 is n-Pr

5. A compound according to claims 1 and 2 which is:

[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*S*-[3α,4β(1*S**,2*R**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester;

[3*S*-[3α,4β(1*R**, 2*S**)]]-3-Hydroxy-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-pyrrolidinecarboxylate, 1,1-dimethylethyl ester;

[3*S*-[3α,4β(1*S**,2*R**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[*N*-Methyl-*N*-(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*R*-[3α,4β(1*R**,2*S**)]]-1-Hydroxyethyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol;

[3*R*-[3α,4β(1*R**,2*S**)]]-4-[7-[(2-Phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-1-(phenylmethyl)-3-pyrrolidinol;

[3*R*-[3α, 4β(1*R**,2*S**)]]-1-Acetyl-4-[7-[(2-phenylcyclopropyl)amino]-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-3-pyrrolidinol.

or pharmaceutically acceptable salts or solvates thereof.

6. A pharmaceutical composition comprising a compound according to any one of claims 1 to 5 in combination with a pharmaceutically acceptable diluent, adjuvent or carrier.

7. A compound according to any one of claims 1 to 5 for use in therapy.

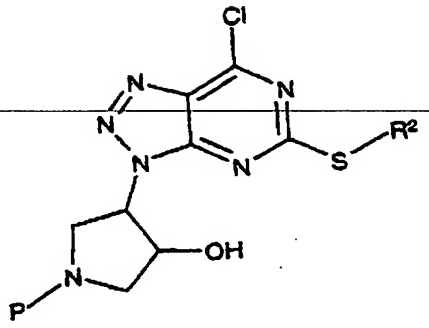
8. A compound according to any one of claims 1 to 5 for use in the treatment or prevention of myocardial infarction, thrombotic stroke, transient ischaemic attacks, peripheral vascular disease and angina.

9. A compound according to any one of claims 1 to 5 for use in the treatment or prevention of angina.

10. A method of treatment of a platelet aggregation disorder which comprises administering to a patient suffering from such a disorder a therapeutically effective amount of compound according to any one of claims 1 to 5.

11. A process for the preparation of a compound of formula (I) which comprises:

a. For compounds of formula (I) where R^1 is H, reacting a compound of formula (II)



(II)

wherein R^2 is as defined in formula (I) and P is a protecting group, with R^3R^4NH, wherein R^3 and R^4 are as defined in (I), and a base and optionally thereafter removing any protecting groups.

b. For compounds of formula (I) where R^1 is CH_2R^5, where R^5 is defined in formula (I), the reaction scheme outlined in a. above is followed by reductive amination using an aldehyde (R^5CHO) and a reducing agent.

c. For compounds of formula (I) where R^1 is COR^6, where R^6 is defined in formula (I), the reaction scheme outlined in a. above is followed by acylation using an acid halide (R^6COX) or anhydride ($(R^6CO)_2O$) or an acid (R^6CO_2H) in the presence of a suitable activating agent.

ABSTRACT

The invention provides novel hydroxypyrrolidine compounds, their use as medicaments, compositions containing them and processes for their preparation.